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PREVENTATIVE/CURATIVE DRUG FOR RESORPTIVE BONE DISEASE
[Kyushusei Kotsu Shikkan Yobo/Chiryozai]

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(Claims)

(Claim 1) A preventative/curative drug for resorptive bone disease, comprising activated protein C and/or prot in C as effective ingredients.

(Claim 2) A preventative/curative drug as defined in Claim 1, wherein said resorptive bone disease is osteoporosis.

(Claim 3) A bone resorption inhibitor, comprising activated protein C and/or protein C as effective ingredients.

(Detailed Explanation of the Invention)

(Field of Use in the Industry) The present invention relates to a preventative/curative drug for resorptive bone disease such as osteoporosis, hypercalcemia caused by malignant tumors, or Paget's disease of the bone, as well as a bone resorption inhibitor, comprising activated protein C (below, called APC) and/or protein C (below, called PC) as effective ingredients.

(Prior Art) Bone tissue ceaselessly continues to turnover, and part of bones that already exists is resorbed by osteoclasts, while on the other hand, new bone is formed by osteoblasts. The forms of bone and bone mass are maintained by this bone metabolic turnover. Bone mass is determined by the functional summation of osteoclasts, which resorb bone, and osteoblasts, which form bone (Hideki Yoshikawa, et al., Nihon Rinsho [*Japanese Clinical Medicine*], 52, 2262-2267, 1994).

Resorptive bone disease is a disease in which the amount of bone resorption by osteoclasts becomes greater than the amount of bone formation by osteoblasts. This results in the reduction of bone mass. Among resorptive bone diseases, there are osteoporosis, malignant

hypercalcemia caused by myeloma and lymphoma, and Paget's diseas of the bone due to local bone resorption. Within osteoporosis, there are regressive osteoporosis and recurrent osteoporosis. Furthermore, of regressive osteoporosis, there are type I osteoporosis recognized after menopause or after ovariectomy, and type II osteoporosis recognized in the elderly. Recurrent osteoporosis is osteoporosis for which the causes are clear, and the causes include chronic arthritic rheumatism, steroid treatments, hyperthyroidism, sexual dysfunction, hyperparathyroidism, acromegaly, diabetes, Cushing's syndrome, Turner syndrome, the taking of immunosuppressants, gastrectomy, renal failure, renal dialysis, *osteogenesis imperfecta*, hypoalkaline phosphatasemia, homocystinuria, malnutrition, limitation of exercise, staying in bed, alcohol toxicity, space flight, and the like. Also, there is also youth-specific osteoporosis for which the cause is not clear. Resorptive bone disease is attracting attention not only as a medical problem, but as a social one, as well. At present, as the aging of members of society is progressing, bone disease has a high frequ ncy of occurrence, causing broken bones all over the body and also leading to the so-called "bedridden elderly."

Osteoclast groups and osteoblast groups are regulated in the expression of their functions by various hormones, growth factors such as cytokine, and extracellular base proteins. As factors which directly inhibit the differentiation and activation of osteoclasts, ther are known calcitonin, prostaglandin, cystine protease inhibitors, C-t rminal parathyroid-related proteins, and the like, and the development of curative drugs for resorptive bone disease such as bisphosphonate,

ipriflavone, and the lik , is advancing.

PC is a protein having vitamin K d pendency, is generated in the liver, and is important as a factor controlling blood clotting. PC inhibits the cascade of clotting by selectively limiting, breaking down, and deactivating activated factor V and activated factor VIII through a complex of thrombin generated in the blood clotting process and thrombomodulin in the *vascular intima* (R. Marlar, et al., *Blood*, 59, 1067-1072, 1982, G.A. Vehar, et al.; *Biochemistry*, 19, 401-410, 1980), and from this fact, the development of PC and APC as anticoagulant drugs is being conducted. Also, with APC, an anti-inflammatory effect is also reported (Kenji Okashima, et al., Abstracts of the 32nd General Conference of the Japan Society of Clinical Hematology, WS111-10, 1990), and as its mechanism of action, the action of inhibiting the activation of white corpuscles, is suggested. Also, it is reported that receptors for APC are expressed in the intimal cells (K. Fukudome, et al., *The Journal of Biological Chemistry*, 269, 26486-26491, 1994), but its expression in osteocytes is not confirmed, nor is there any report that record d its having an effect on bone tissue.

(Problems the Invention Attempts to Solve) To date, there have been used as curative drugs for resorptive bone disease such as osteoporosis: estrogen, which is a female hormone and which promotes osteogenesis; activated vitamin D_3, which ameliorates the reduction of bone mass; calcium agents, which are used as supplemental treatments; calcitonin, which has the effect of lowering serum Ca with a peptide hormone secreted from the thyroid gland; elcatonin, which is a derivative of calcitonin; and ipriflavone, which reinforces the effect

of estrogen, and the like. However, estrogen is contraindicated for patients who are at risk of ovarian hemorrhage, thrombosis from long-term use, and estrogen-dependent tumors such as in ovarian cancer. Also, with activated vitamin D_3, calcitonin, elcatonin, and ipriflavone, there are reports of side effects such as ill temper, vomiting and like gastrointestinal disorders, shock, and hypersensitivity, and their effects are not necessarily satisfactory. Also, from the factor that osteoporosis is a disease having a long period of treatment, the development of a safe curative drug having light side effects is an important issue.

(Means for Solving the Problems) In the course of studying the physiological effects of APC on various cells, the inventors have discovered that APC acts on osteocytes and inhibits bone resorption, and by further accumulating devoted research, the present invention was completed.

The present invention is explained in detail below. The first mod of the present invention is a preventative/curative drug for resorptiv bone disease comprising APC and/or PC as active ingredients. In resorptive bone disease, there are included osteoporosis, malignant hypercalcemia caused by myeloma and lymphoma, and Paget's diseas of the bone due to local bone resorption.

The second mode of the present invention is a preventative/curative drug for osteoporosis, comprising APC and/or PC as active ingredients. In osteoporosis, there are included regressive osteoporosis, recurrent osteoporosis, and youth-specific osteoporosis. Furthermore, in regressive osteoporosis, there are included type I osteoporosis, which

is recognized after menopause or after ovari ctomy, and type II osteoporosis, which is recognized in the elderly. Also, as causes of recurrent osteoporosis, there can be mentioned chronic arthritic rheumatism, steroid treatments, hyperthyroidism, sexual dysfunction, hyperparathyroidism, acromegaly, diabetes, Cushing's syndrome, Turner syndrome, the taking of immunosuppressants, gastrectomy, renal failure, renal dialysis, *osteogenesis imperfecta*, hypoalkaline phosphatasemia, homocystinuria, malnutrition, limitation of exercise, staying in bed, alcohol toxicity, space flight, and the like.

The third mode of the present invention is a bone resorption inhibitor, comprising APC and/or PC as active ingredients. Its usefulness is expected in promoting healing after bone breaks, the prevention/cure of broken bones due to fatigue, and raising the rate of acceptance of bone transplants. Also, it is useful as an experimental drug for research.

The APC used in the present invention is a protein having the property of selectively limiting, breaking down, and deactivating activated factor V and activated factor VIII in the cascade of blood clotting. Also, PC, which is converted into APC by a thrombin-thrombomodulin complex in the body, is expected to have the same effect as APC in the preventative/curative drug of the present invention. Accordingly, APC and/or PC may be either natural or produced by genetic engineering, and modified forms obtained by genetic engineering methods are also acceptable. When used as a medical drug, preferably natural human APC and/or PC is/are desired. More preferably, natural human APC is desired. Of such PC, in regard to natural types, there can be

mentioned PC manufactured from human plasma by the method of W. Kisiel (*Journal of Clinical Investigation*, 64, 761-769, 1979), and PC disclosed in the publication of Japanese Laid-Open Patent No. 61-205487, the publication of Japanese Laid-Open Patent No. 62-111690, as well as PC derivatives disclosed in the publication of [European patent] No. EP443875, Japanese Laid-Open Patent No. 4-211380, and the like, as recombinant gene types. Also, although there is no particular restriction in the method of activating PC, it can be implemented, for example, by the method of activation by thrombin (W. Kisiel, *Biochemistry*, 16, 5824-5831, 1977), and the method of activation by fixed thrombin (publication of Japanese Laid-Open Patent No. 3-93799). Also, it can also be implemented by methods of activation by snake venom or synthetic peptides, and the like.

APC, in regard to natural types, is sold commercially on the market, for example, as protein C, activated human (Serbio Laboratory Co.) being a freeze-dried product manufactured from human plasma, or as purified APC (Diagnostica Stago Co.). In regard to recombinant gene types, there can be mentioned active protein C as disclosed in the publication of Japanese Laid-Open Patent No. 62-111690, or activated human protein C as disclosed in the publication of Japanese Laid-Open Patent No. 3-72877.

APC and/or PC are presently anticoagulants under clinical development, there are few side effects, including a tendency for hemorrhage as accompanies heparin, and highly safe medical drugs are expected. Actually, for APC from human blood, its safety has been confirmed by intravenous toxicity tests in mice, general pharmacological

tests, and viral deactivation tests (publication of Japanese Laid-Open Patent No. 6-183996, and Kumiko Aoki, et al., Oyo Yakuri [*Applied Pharmacology*], 48, 239-250, 1994). Also, with heparin and low molecular-weight heparin, it is known that there is a reduction of bone mass by its use (Masahiko Nishiyama, et al., Abstracts of the 68th Annual Conference of the Japanese Pharmacological Society, $P_2$293, 1995). Meanwhile, from the fact the APC and/or PC used in the present invention inhibit bone resorption, APC and/or PC differ from heparin and low molecular-weight heparin in that they are anticoagulants having the effect of increasing bone density.

APC and/or PC used in the present invention have the effects of inhibiting bone resorption. Accordingly, they can be used as preventative/curative drugs for various resorptive bone diseases, for example, osteoporosis, Paget's disease of the bone, and hypercalcemia subsequent to malignant tumors, and the like. Also, they can be used in collagen diseases that are centered on chronic arthritic rheumatism, and progressive bone embrittlement. Accordingly, the drugs can be used for the purpose of preventing/curing or improving, for example, bone destruction in chronic arthritic rheumatism or the reduction of bone mass which is one side effect of steroid treatments, and furthermore the promotion of cure of these diseases is expected. Furthermore, from the fact that the APC and/or PC used in the present invention inhibit bone resorption, the promotion of healing after bone breaks in various places, the prevention/cure of broken bones by fatigue, or raising the rate of acceptance of bone transplants are expected.

The drug preparation of the present invention should contain APC

and/or PC, and it can be manufactured by all pharmaceutical manufacturing methods. Said APC and/or PC can be prepared with any suitable carrier or medium generally used as a drug, for example, sterilized water or physiological saline solution, vegetable oil, mineral oil, high-grade alcohol, high-grade fatty acid, a harmless organic solvent, and the like, and may be further combined according to need with an excipient, coloring agent, emulsifier, suspension agent, surfactant, solubility aid, anti-adhesive, stabilizer, preservative, moisturizer, antioxidant, buffer, isotonicizer, pain reliever, and the like, into a drug preparation such as an injectable drug, nasal absorbent, oral drug, or embedded preparation; most preferably an injectable drug. Several specific examples of these are well known. As an injectable drug, it can be provided, for example, as a freeze-dried product, an injectable liquid, or in a form sealed in an infusion pump. Also, as a time-released preparation, it can also be taken in a form contained inside a microcapsule, microsphere, ribosome or nanosph re, or as a preparation containing a polymer base.

The preparation of the present invention can be taken by methods such as, for example, intravenous injection, subdermal injection, intramuscular injection, and in-joint injection, in a quantity of about 1-2000μg/kg, preferably about 10-1000μg/kg as quantity of APC and/or PC protein, and it can be taken continuously by keeping it attached to the body as an embedded preparation or sealed in an infusion pump, and the like.

(Experimental Examples) In order to explain the present invention in detail, the effects are explained specifically below according to

experimental examples. However, the present invention is by no means limited to these.

(Experimental Example 1: Effect of human-originating APC on bone resorption) After removing the soft tissue, a mouse shin bone and thigh bone were thinly sliced in an α-MEM medium and further mixed with a touch mixer, and then it was set aside and the obtained supernatant was used as the total osteocytes including osteoclasts. Next, following the method of Y. Takeda, et al. (*Bone and Mineral*, 17, 347-357, 1992), the effect of the tested drug on the formation of resorption bits by the osteoclasts on slices of ivory was examined. The ivory was cut into round ivory slices having a thickness of about 150μ and a diameter of about 6mm, the total osteocytes including the osteoclasts were applied and incubated for 2 hours at 37°C, then the adhered cells were removed, and the α-MEM medium was added and cultured for 5 days. On the fifth day, 0.25M of ammonia was added and the reaction was stopped, and then the cells adhered to the ivory slices were removed by ultrasound processing. The surface area of the formed resorption pits was dyed with acidic hematoxylin, and then it was measured under a microscope. The effect of the tested drug was computed as the [percentage] rate of inhibition according to differences in the surface area of resorption pits of the group not having the tested drug added and the group having the tested drug. The results are shown in Figure 1 and Figure 2 [sic: no Figure 2 included]. As is clear from Figure 1, APC and elcatonin inhibited the formation of resorption pits on the ivory slices dependent on volume. This formation of resorption pits on ivory slices is an indicator of bone resorption, and it is inhibited by drugs used for

resorptive bone diseas , such as elcatonin. Consequently, APC is believed to be a preferable drug for its preventative/curative effect on resorptive bone disease.

(Embodiments) The present invention is explained more specifically by presenting embodiments below, but the present invention is not limited to these.

(Embodiment 1)

APC	10mg
Refined gelatin	20mg
Sodium phosphate	34.8mg
Sodium chloride	81.8mg
Mannitol	25mg

The above ingredients were dissolved in 10ml distilled water for injection, and after having been filtered for bacteria, it was apportioned into 1.0ml sterile vials and freeze-dried to prepare an injectable preparation.

(Embodiment 2)

APC	50mg
Albumin	20mg
Sodium phosphate	34.8mg
Sodium chloride	81.8mg
Mannitol	25mg

The above ingredients were dissolved in 10ml distilled water for injection, and after having been filtered for bacteria, it was apportioned into 1.0ml sterile vials and freeze-dried to prepare an injectable preparation.

(Effect of the Invention) The preventative/curative drug having APC and/or PC as active ingredients of the present invention has the effect of inhibiting bone resorption. Accordingly, it can be used as a preventative/curative drug for various diseases in which bone resorption is advanced, for example, resorptive bone diseases such as osteoporosis, malignant hypercalcemia, Paget's disease of the bone, and reduction of bone due to chronic arthritic rheumatism, and as a bone resorption inhibitor for promotion of healing after bone breaks and as a test drug for research. Also, the APC and/or PC of the present invention are expected to have fewer side effects compared with already-existing drugs, and they should serve as safe curative drugs for which long-term use is possible. Furthermore, because the APC and/or PC of the present invention have few side effects, such as a tendency for hemorrhage and the reduction of bone mass which follow treatment by heparin and low molecular-weight heparin, they have a higher level of safety when used as anticoagulants, and as dialysis drugs, as well.

(Brief Explanation of the Drawing)

(Figure 1) is a graph showing the effects of APC used in the present invention and elcatonin in inhibiting formation of resorption pits in ivory.

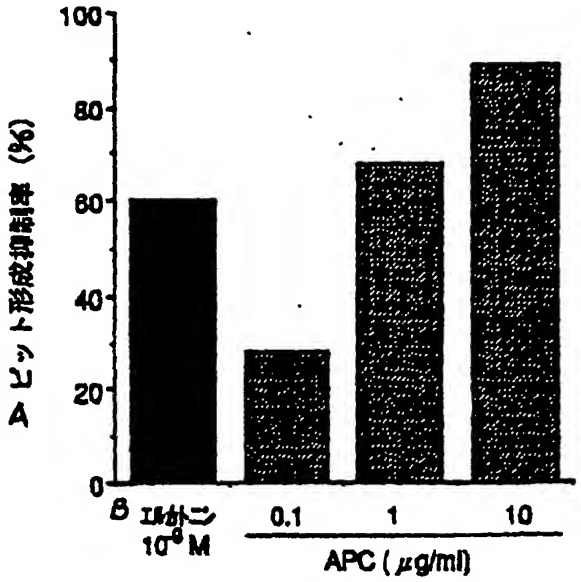

Figure 1

[Key:]
A. rate of inhibition of pit formation (%); B. elcatonin